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International application number: PCT/US05/002261

International filing date: 24 January 2005 (24.01.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/539,063
Filing date: 23 January 2004 (23.01.2004)

Date of receipt at the International Bureau: 21 February 2005 (21.02.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

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APPLICATION NUMBER: *60/539,063*
FILING DATE: *January 23, 2004*
RELATED PCT APPLICATION NUMBER: *PCT/US05/02261*

Certified by

W. Dudas

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EL084752599US

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Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

AN EFFICIENT METHOD TO SYNTHESIZE BENZYL GROUP-PROTECTED ALPHA-PENTAGALLOYLGLUCOSE...

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ENCLOSED APPLICATION PARTS *(check all that apply)*

[X] Specification *Number of Pages* 5 — [ ] CD(s), Number _____

[X] Drawing(s) *Number of Sheets* 2 — [ ] Other (specify) _____

[ ] Application Date Sheet. See 37 CFR 1.76

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[X] Applicant claims small entity status. See 37 CFR 1.27.

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[ ] No.

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Respectfully submitted,

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Date January 23, 2004

REGISTRATION NO. 50,627 *(if appropriate)*

Docket Number: 27211/04095

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

Express Mail Label No. EL084752599US

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AN EFFICIENT METHOD TO SYNTHESIZE BENZYL GROUP-PROTECTED

CERTIFICATE OF EXPRESS MAILING

Date of Deposit: January 23, 2004

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27211/04095

AN EFFICIENT METHOD TO SYNTHESIZE BENZYL GROUP-PROTECTED ALPHA-PENTAGALLOYLGLUCOSE (α-PGG) AND ITS ANALOGUES

BACKGROUND OF THE INVENTION

[0001] α-PGG has been shown to possess anti-diabetic and other bioactivites that make it a target for the development of new drugs. Since is does not occur in nature, is must be prepared by a multi-step synthesis. Currently known procedures to synthesize α-PGG comprise two main steps: an initial acylation reaction generates benzyl group-protected α-PGG. This precursor must be isolated in order to obtain pure α-PGG in the final hydrogenation reaction. The first step (acylation) produces large amounts of side products that are very difficult to remove. The most important of these unwanted chemicals are the β-isomer of the PGG precursor, dialkyl urea, and N-acylurea derivatives. Chromatography, which is quite expensive, is the only technique that allows for the purification of benzyl-group protected α-PGG. Only gram quantities of the target compound can be produced. The high costs of chromatography and the difficulties in scale-up procedures suitable for producing kilogram to ton quantities of product preclude industrial application of the procedure.

[0002] Accordingly, a need exists for better methods of producing the precursors of α-PGG. The new methods should eliminate the need for chromatography, and should be amenable to scaling up to kilogram or ton quantities.

SUMMARY OF THE INVENTION

[0003] The present invention provides a new method to synthesize a key precursor of alpha-pentagalloylglucose (α-PGG). The new process reduces the costs for synthesis of α-PGG by more than 60%. The method allows for the production of the precursor on a kilogram to ton scale. Moreover, it can be used to efficiently synthesize analogues of α-PGG that are modified in the carbohydrate or acyl parts of the molecule.

[0004] The method of the present invention comprises the steps of: (a) suspending a highly reactive acylation agent and α-D-glucose or another analogue in a donor solvent; (b) adding DMAP to the suspension and stirring until the highly reactive acylation agent is dissolved; (d) reacting the mixture at room temperature while evaporating off solvent to form a residue; (e) taking up the residue in an appropriate solvent; (f) filtering the residue and solvent mixture; and (g) evaporating off the solvent.

[0005] A preferred class of highly reactive acylation agents are acid chlorides.

[0006] Some suitable α-D-glucose analogues are those in which the analogue is selected from hexoses, pentoses, and tetroses, or wherein the ring oxygen is replaced by a carbon, nitrogen or sulfur

[0007] Preferred solvents are those that produce an $\alpha:\beta$ ratio of at least 90:10; more preferred solvents are those that produce an $\alpha:\beta$ ratio of at least 95:10. Some preferred solvents include acetonitrile, 1,4-dioxane and THF. The most preferred solvent is acetonitrile.

[0008] After the addition of DMAP, the mixture is stirred at room temperature until the acylation agent is dissolved, generally about 5 to 10 minutes.

[0009] The mixture is generally allowed to react at room temperature until the solvent has evaporated off. The preferred solvent for taking up the residue in step (e) is toluene, heated to about 60°C. When a heated solvent is used, the solvent and residue mixture are allowed to cool to room temperature prior to the filtration step.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] **Figure 1** illustrates the state of the art process for synthesizing benzyl group protected α-PGG.

[0011] **Figure 2** illustrated the synthetic scheme of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[0012] Known methods for synthesizing the α-PGG precursor rely on a procedure that uses a carbodiimide coupling agent, such as DCC, in conjunction with *N,N*-dimethylaminopyridine (DMAP) as a catalyst. See, *e.g.*, *Tetrahedron*, Vol. 53, No. 31, pp. 10725-10732 (1997). This procedure is suitable only for small-scale synthesis of the α-PGG precursor because of the large number of side products produced. Additionally, a time consuming and expensive chromatographic purification step is necessary. In addition, the carbodiimide reagents used in the process are strong sensitizers, which would pose a severe health risk for the operators of large-scale production facilities.

[0013] The present invention provides new methods of producing the key precursor of α-PGG using different reagents than conventional methods. The reagents used in the new methods are used in smaller quantities than the amounts of reagents used in previous methods. The new methods also eliminate the need for chromatography to remove side products. In addition, the new methods can be run at room temperature, avoiding costs incurred by heating and/or cooling the reaction mixture. Overall, the new process is highly cost efficient, and it allows for synthesis of ton quantities of the α-PGG precursor. Moreover, it allows for synthesis of analogues of benzyl-group protected α-PGG that are modified in the carbohydrate or acyl parts of the molecule.

[0014] Figure 1 shows the state of the art synthetic scheme for the production of benzyl group protected α-PGG. The final step chromatographic step is very expensive and precludes production on an industrial scale. A complete separation of the α- and β-isomers of the benzyl group-protected PGG is extremely difficult. Figure 2 shows the synthetic scheme of the present invention. With the present invention, the costly chromatography step is unnecessary. The small

amounts of β-isomer that are formed may easily be removed after the final steps in the PGG synthesis, removal of the benzyl groups by hydrogenation. The yield of the method is almost quantitative.

[0015] For the first step of production of α-PGG and its analogues (acylation) highly reactive acylation agents, such as acid chlorides, are used. In addition, the solvent used for the reaction is chosen to favor formation of the α-isomer and suprress formation of the β-isomer of the PGG, or PGG analogue, precursor. Preferably, the solvent chosen yields an α:β ratio of greater than 90:10. More preferably, the solvent chosen yields an α:β ratio of greater than 95:5. Suitable sovents include donor solvents such as, but not limited to, acetonitrile, 1,4-dioxane, and tetrahydrofuran. Acetonitrile is the preferred solvent. Moreover, the methods of the present invention produce no dialkylurea or N-acylurea side products during the reaction process. Additionally, the yields of the reaction is almost quantitative, greater than 95%.

[0016] The coupling of the benzyl-group protected gallic acid with the α-D-glucose starting material to obtain the precursor of α-PGG and its analogs is performed using a highly reactive acylating agent, such as an acid chloride. This ensures that α-D-glucose reacts with the acylating agent before it can rearrange to β-D-glucose. This, in turn, avoids the formation of the β-isomer of the PGG precursor, or PGG analogue precursor. The choice of a solvent, such as acetonitrile, that yields a high α:β ratio is a second factor that helps to achieve a very high α:β ratio in the products.

[0017] Additionally, the use of an acid chloride in the reaction, makes it unnecessary to use a carbodiimide coupling agent. This, in turn, avoids the formation of the dialkylurea and N-acylurea side products. This eliminates the need for chromatographic purification.

[0018] The present invention also provides methods of making precursors of α-PGG analogues. The methods of the present invention are useful for making analogues of α-PGG in which the glucose part of the PGG is substituted by other sugars, preferably hexoses, pentoses, or tetroses. Preferred hexoses include galactose, mannose, idose, talose, altrose, allose, gulose, fructose, or similar. Preferred pentoses include xylose, ribose, arabinose, and lyxose. Preferred tetroses include threose and erythrose. The methods of the present invention are also useful for making

analogs in which the glucose part of the PGG is substituted by sugar analogues, of glucose, other hexoses, pentoses, or tetroses, in which the ring oxygen of the sugar analogue is substituted by carbon, nitrogen, or sulfur. The methods of the present invention are also useful for making analogues wherein the gallic acid part of the PGG is replaced by other phenols. Preferred phenols include, but are not limited to 2,3-dihydroxybenzoic acid, 3,4-dihydroxybenzoic acid, and 3,5-dihydroxybenzoic acid. Those skilled in the art would be able to recognize other modifications of the α-PGG precursor that could be prepared by the methods of the present invention.

[0019] **Materials and Methods**

[0020] The names and Chemical Abstracts register numbers of the benzyl group-protected PGG's are: α-D-glucopyranose pentakis [3,4,5-tris(phenylmethoxy)benzoate] (CA Reg. No. 70424-95-2) and β-D-glucopyranose pentakis [3,4,5-tris(phenylmethoxy)benzoate] (CA Reg. No. 122625-60-9).

[0021] The acid chloride (459 mg, 1.0 mmol) and finely powdered α-D-glucose (36.0 mg, 0.2 mmol) were suspended in 10 mL acetonitrile at room temperature. DMAP (128 mg, 1.05 mmol) was added last, and the mixture was stirred at room temperature. The chloride dissolved within 5 to 10 minutes after the addition of DMAP. After 18 hours at room temperature, the solvent was evaporated. The residue was taken up in 5 mL of toluene at 60°C. After cooling to room temperature, the solution wais filtered through a layer of silica gel (250 mg, 1.2 cm thick). The silica gel was washed with 5 mL of a mix of toluene and ethyl acetate (100:4) to elute any product that may have stuck to the silica gel. The solvent was evaporated. The product is obtained as highly viscous oil after drying it in an oil pump vacuum for 5 hours. The yield was 455 mg (99%).